

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
21 June 2001 (21.06.2001)

PCT

(10) International Publication Number  
**WO 01/44486 A1**

(51) International Patent Classification<sup>7</sup>: **C12P 7/26,**  
7/28, C07C 45/45, 49/245

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(21) International Application Number: PCT/AU00/01543

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(22) International Filing Date:

13 December 2000 (13.12.2000)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

PQ 4625 13 December 1999 (13.12.1999) AU

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,  
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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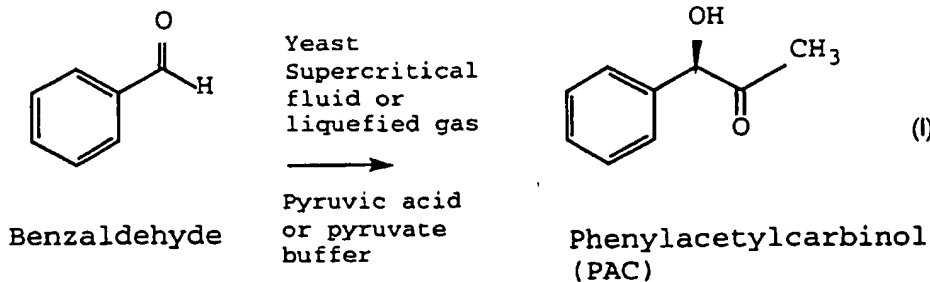
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Published:

— With international search report.

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: YEAST-BASED PROCESS FOR PRODUCTION OF L-PAC



(57) Abstract: The invention provides a method of synthesis of a substituted or unsubstituted carbinol compound, comprising the steps of subjecting the corresponding substituted or unsubstituted aromatic aldehyde to acyloin condensation mediated by yeast in the presence of either (a) a supercritical fluid or (b) a liquefied gas, and recovering the carbinol compound. Preferably the yeast is *Saccharomyces cerevisiae*. In a particularly preferred embodiment the aromatic aldehyde is benzaldehyde and the carbinol is phenylacetylcarbinol, according to the reaction (I): in which the benzaldehyde, the pyruvic acid, or both may optionally be substituted.

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YEAST-BASED PROCESS FOR PRODUCTION OF L-PAC

This invention relates to organic compounds useful as precursors for the synthesis of a variety of products, particularly for synthesis of compounds useful as pharmaceutical agents. The method of the invention utilises yeast-mediated catalysis in the presence of a supercritical fluid or liquefied gas, and in particular the yeast-mediated condensation between pyruvate and a substituted aromatic aldehyde to yield the corresponding acyloin (hydroxy ketone) compound. In a preferred embodiment, the reaction is that between pyruvate and benzaldehyde to yield phenylacetylcarbinol, the precursor to ephedrine, in high enantiomeric purity.

BACKGROUND OF THE INVENTION

Physicochemical methods for production of enantiomerically pure compounds usually involve multi-step synthesis incorporating one or more steps which are asymmetric, and laborious purification procedures. Such methods are not only tedious, but frequently provide relatively poor yields. Alternatively enantiomerically-pure starting materials can be used, together with enantioselective reaction steps; however, such pure starting materials are available only for a very limited number of desired compounds.

In an attempt to overcome the difficulties of using traditional organic chemical methods, biological systems have been intensively investigated. Such systems show a very high degree of stereoselectivity in their reactions, and therefore microbiological, enzymatic or chemoenzymatic reactions for achieving specific reaction steps with a variety of reagents have been attempted. For example, microorganisms of a number of genera have been proposed for synthesis of optically active  $\alpha$ -substituted derivatives of 3-hydroxypropionic acid for use as intermediates in the synthesis of compounds such as  $\alpha$ -tocopherol, muscones and pharmaceutical, insecticidal and

agricultural chemical agents (U.S. Patent No. 4734367 by Hoffman-La Roche, Inc.). Most such procedures use whole-cell fermentation systems in aqueous media, or isolated enzymes with a specific desired activity. However,

5 fermentation systems present the disadvantage that purification of the desired product can be difficult, and yields tend to be low; while the yield and convenience of the reaction can be improved by utilising immobilised cells, or cells which have been selected or genetically

10 modified, this adds significantly to the cost of the process. The use of purified enzymes is normally prohibitively expensive, and again without the use of immobilised enzymes the yields tend to be low and purification difficult.

15 In recent years, intense efforts have been directed towards development of methods which are highly selective, provide a good rate of transformation, and enable easy, non-chromatographic separation and purification of the product. It would be particularly

20 desirable if reactions could be carried out in organic solvents, since these are particularly convenient for large scale reactions and purifications.

It has been shown that dry baker's yeast is able to effect non-fermentative reduction of  $\alpha$ -keto esters in

25 organic solvents such as hexane or benzene, to produce the corresponding  $\alpha$ -hydroxy esters with good yield and selectivity (Nakamura *et al*, 1988; Nakamura *et al*, 1990; Nakamura *et al*, 1991; Nakamura *et al*, 1993); reduction of  $\beta$ -keto esters in petroleum ether, diethyl ether, toluene,

30 carbon tetrachloride and petrol has also been demonstrated (Jayasinghe *et al*, 1993; Jayasinghe *et al*, 1994; North, 1996). Although initially it was thought that immobilisation of yeast, for example in polyurethane, was essential in order to maintain stability of cell membrane-

35 bound coenzymes for the dehydrogenases and reductases which catalyse the reaction (Nakamura *et al*, 1988; Nakamura *et al*, 1990), it was subsequently found that the addition of a

very small proportion of water to the organic system would avoid the need for immobilisation (Nakamura et al, 1991).

- Ephedrine ( $\alpha$ -[1-(methylamino)ethyl]benzene-methanol), originally isolated from plants of the genus *Ephedra*, occurs as the naturally-occurring isomers 1-ephedrine and d-pseudoephedrine, and other pharmacologically active isomers include d-ephedrine and l-pseudoephedrine. These compounds are adrenergic sympathomimetic agents and have antihistamine activity; 1-ephedrine is widely used as a bronchodilator, while d-pseudoephedrine is widely used as a decongestant. Compounds of these groups are present in a very wide range of prescription and over-the-counter pharmaceutical formulations.
- The production of 1-phenylacetylcarbinol, a precursor of 1-ephedrine, by catalysis using whole baker's yeast cells in aqueous medium was one of the first microbial biotransformation processes to be used commercially (Neuberg and Hirsch, 1921; see also Hildebrandt and Klavehn, 1934). This reaction involves the yeast-induced condensation of benzaldehyde with acetyl-coenzyme A. The reaction has been widely investigated, and has been shown to be mediated by the enzyme pyruvate decarboxylase (Groger, Schmander and Mothes, 1966). It has also been shown that the reaction has a relatively broad specificity for the substrate, enabling a variety of substituted aromatic aldehydes to be converted to the corresponding substituted optically-active phenylacetylcarbinols (Long, James and Ward, 1989).
- Although this yeast-catalysed system has been widely exploited, this has normally utilised aqueous systems, which are inconvenient for large-scale extraction and purification, which require organic solvents. Additionally, fermentation systems present the disadvantage that purification of the desired product can be difficult, and yields tend to be low; while the yield and convenience of the reaction can be improved by utilising immobilised

cells, or cells which have been selected or genetically modified, this adds significantly to the cost of the process. The use of purified enzymes is normally prohibitively expensive, and again without the use of  
5 immobilised enzymes the yields tend to be low and purification difficult.

In our earlier International Application PCT/AU99/00433, we showed that yeast-mediated acyloin condensation of benzaldehyde can be achieved in an organic  
10 solvent using non-fermenting yeast. The formation of side-products was suppressed by an addition of a small proportion of ethanol to the reaction mixture and by conducting the reaction at reduced temperature. When using organic solvents, there are problems with associated  
15 toxicity, occupational health and safety issues, flammability and waste disposal/recycling.

We have now surprisingly found that the yeast-mediated acyloin condensation of benzaldehyde can be achieved in supercritical or liquefied carbon dioxide or in  
20 liquefied petroleum gas. This reaction results in superior conversion of the aromatic aldehydes to the desired carbinol. In a preferred embodiment, yields of around 79% with the total absence of side-products were obtained using the method of the invention.

25 Even more surprisingly, it was found that carbon dioxide in either liquid or supercritical form deactivates the reduction reaction, thus inhibiting the formation of undesired side-products.

Since carbon dioxide is non-toxic and can be  
30 readily recycled, this method avoids the problems associated with reactions involving organic solvents.

Other supercritical fluids may be used, but may not prevent the formation of side-products without the addition of an inhibitor and may not have the same  
35 environmental advantages.

SUMMARY OF THE INVENTION

In a first aspect, the invention provides a method of synthesis of a substituted or unsubstituted carbinol compound,

5 comprising the steps of subjecting the corresponding substituted or unsubstituted aromatic aldehyde to acyloin condensation mediated by yeast in the presence of either (a) a supercritical fluid, or (b) a liquefied gas, and recovering the carbinol compound.

10 Any yeast capable of effecting the acyloin condensation reaction may be used. It is economically advantageous to use the cheapest yeast available, and ordinary baker's yeast, *Saccharomyces cerevisiae*, is preferred. Strains of yeast adapted to other purposes,  
15 including brewing yeast and wine or sherry yeasts could also be employed. Strains specifically adapted to a supercritical fluid environment or for enhanced acyloin condensation efficiency may be used; such strains include conventionally-selected and genetically modified strains.  
20 For maximum efficiency of reaction, it is advisable to present the maximum surface area of yeast for contact with the reactants. This can be effected by using "active" dried yeast, which is readily commercially available as "instant dry yeast", and may be stored at room temperature.  
25 Alternatively, well-pulverised dry baker's yeast may be used. Other yeasts, such as those described in U.S. Patent No. 4734367, or fungi such as those disclosed in Chênevert et al (1992) may also be used. The person skilled in the art will readily be able to test whether any specific  
30 organism will function for the purposes of the invention, using the methods described herein.

The supercritical fluid may be any suitable supercritical fluid with a critical temperature below 50°C. We have found that carbon dioxide is particularly suitable,  
35 as the reaction can be performed at a moderately elevated temperature, suitably between 33 to 42°C, preferably 35°C.

At these temperatures, the corresponding pressure may range between 1500 to 2500 psi, preferably 1500 psi.

Other suitable supercritical fluids are known in the art, for example:

5

Fluid	Critical temperature (°C)	Critical pressure (psi)
Ethane	32.4	707.8
Nitrous oxide	36.6	1050
Xenon	16.7	847
Fluoroform (CHF <sub>3</sub> )	26.3	705
Monofluoromethane	42	812.2
Sulphur hexafluoride	45.7	545.3
Chlorotrifluoromethane	29	561.3

When the condensation is performed in the presence of a supercritical fluid, the carbinol compound is recovered by subjecting the reaction mixture to extraction  
10 with a supercritical fluid or an organic solvent such as ethylacetate or diethylether.

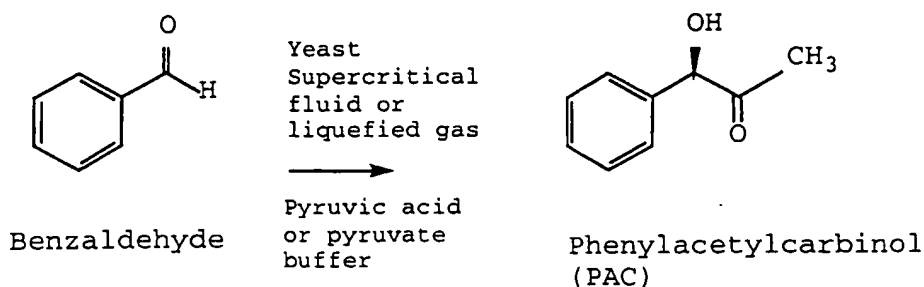
The liquefied gas may be carbon dioxide, a hydrocarbon such as methane, ethane, propane, butane, ethylene, or the like, or mixtures thereof. Liquefied  
15 petroleum gas may be used.

The person skilled in the art will readily be able to test whether any specific supercritical fluid will function for the purposes of the invention, and to identify conditions of suitable temperature and pressure, using the  
20 methods described herein.

The person skilled in the art will also readily be able to determine whether a particular supercritical fluid or liquefied gas effectively inhibits the formation of side-products, or whether the addition of a suitable  
25 inhibitor is necessary. The use of ethanol as a suitable inhibitor is discussed in our International Application PCT/AU99/00433.

Once the yeast-mediated reaction has been completed, the system is de-gassed and the yeast extracted with either a supercritical fluid or an organic solvent.

In a preferred embodiment, the invention provides  
5 a method for yeast-mediated conversion of benzaldehyde to phenylacetylcarbinol, according to the following reaction:



10 It will be clearly understood that the benzaldehyde, the pyruvic acid, or both may optionally be substituted, and that pyruvate, for example sodium pyruvate, may be used as an alternative to pyruvic acid. As a further alternative, a precursor of pyruvic acid which  
15 can be converted *in situ* to pyruvic acid may be used, for example, lactic acid, unless the supercritical fluid or liquefied gas is carbon dioxide. Aromatic aldehydes substituted with alkyl, aryl, halo, nitro, hydroxy, alkoxy, amino, carbonyl, thioxy or thioalkoxy groups or composites  
20 of these groups may also be used instead of benzaldehyde.

For either sodium pyruvate or pyruvic acid, the pH of the pyruvate/citrate buffer solution is preferably between 5 and 6, more preferably pH 6. Between 0.6 and 1.2 ml buffer/g of yeast should preferably be used for  
25 optimal results.

While the ratio of yeast to substrate will vary depending on the individual system, and is readily determined experimentally using routine trial and error methods, we have found that for the conversion of  
30 benzaldehyde to phenylacetylcarbinol the optimum ratio is 4.2 g yeast/mmol benzaldehyde; increasing the amount of



yeast results in only a small increase in conversion, and lower amounts of yeast provide lower conversion.

Similarly, the optimum reaction time may readily be determined, and for the benzaldehyde-phenylacetyl-  
5 carbinol system we have investigated reaction times from 3 to 24 hours. Reactions longer than 24 hours do not lead to higher yields.

The reaction is significantly faster than prior art methods, even those using yeast. The supercritical  
10 fluid or liquefied gas used in the process can be recycled. The yeast can be used for other purposes, for example in animal feed, especially when this fluid or gas is carbon dioxide.

#### 15 DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only to the following non-limiting examples.

#### 20 Example 1: Yeast-Mediated Acyloin Condensation of Benzaldehyde Using Sodium Pyruvate

Benzaldehyde (0.010 g, 0.1 mmol), sodium pyruvate (0.205 g), baker's yeast (0.415 g) and citrate buffer (0.415 ml, pH 6) was placed into a 15 ml stainless steel  
25 vessel. The vessel was pressurised to 2000 psi by pumping dried liquid carbon dioxide via a HPLC pump into the vessel and stirred in a 33°C water bath for 24 hours. The reaction vessel was cooled after this time to room temperature and slowly de-gassed. The vessel contents and residue were  
30 washed 3 times with diethylether and filtered.

Gas chromatography analysis revealed that 82% of phenylacetylcarbinol had formed.

The mixture was purified using radial chromatography with petroleum ether : diethylether (70:30)  
35 to give pure 1-PAC.

$[\alpha]_D = -377.7$  ( $c = 0.006$ ,  $\text{CHCl}_3$ )

$^1\text{H NMR}$  ( $\text{CDCl}_3$ )

$\delta 7.30\text{--}7.55$ , m, Ph;  $\delta 5.15$ , s, CH;  $\delta 2.10$ , s,  $\text{CH}_3$ .

5 Example 2:      Yeast-Mediated Acyloin Condensation of  
                         Benzaldehyde Using Sodium Pyruvate

                 Benzaldehyde (0.010 g, 0.1 mmol), sodium pyruvate  
(0.205 g), baker's yeast (0.415 g) and citrate buffer  
(0.415 ml, pH 6) was placed into a 15 ml stainless steel  
10 vessel. The vessel was pressurised to 2000 psi by pumping  
dried liquid carbon dioxide via a HPLC pump into the vessel  
and stirred in a 33°C water bath for 4 hours. The reaction  
vessel was cooled after this time to room temperature and  
slowly de-gassed. The vessel contents and residue were  
15 washed 3 times with diethylether and filtered.

                 Gas chromatography indicated a 62% conversion to  
phenylacetylcarbinol had formed.

                 The mixture was purified using radial  
chromatography with petroleum ether : diethylether (70:30)  
20 to give pure l-PAC.

$[\alpha]_D = -377.7$  ( $c = 0.006$   $\text{CHCl}_3$ )

lit:             $[\alpha]_D = -408.7$  ( $c = 1.1$   $\text{CHCl}_3$ )

Takeshita, M. and Sato, T., Chem. Pharm. Bull. 1989 37 1085

$^1\text{H NMR}$  ( $\text{CDCl}_3$ )

25             $\delta 7.30\text{--}7.55$  m, Ph;  $\delta 5.15$ , s, CH;  $\delta 2.10$ , s,  $\text{CH}_3$ .

                 Conducting the above reaction at different  
temperatures and pressures for 4 hours gave the following  
conversions to  
l-PAC:

30

Pressure → Temperature ↓	1500 psi	2000 psi	2500 psi
33°C	63%	62%	38%
35°C	68%	45%	79%
38°C	47%	41%	76%
42°C	10%	12%	36%

Example 3: Comparative data

The following is a comparison of the supercritical fluid system (using carbon dioxide) with the organic solvent system disclosed in our earlier application.

	Supercritical fluid (CO <sub>2</sub> )	Organic solvent
conversion	79%	32%
time	4 hours	24 hours
g yeast / mmol benzaldehyde	4.2	5
ml buffer / g yeast	1	1
g sodium pyruvate. / mmol benzaldehyde	2	2.5
temperature	35°C	5°C
pressure	2500 psi	N/A

Example 4: Reaction in supercritical carbon dioxide

Benzaldehyde (0.137g, 1.3mmol), sodium pyruvate (2.168g, 19.7mmol), pH 6 citrate buffer (5.4ml) and yeast (5.4g) were placed into a 250ml stainless steel pressure vessel. This vessel was pressurised to 1500 psi by pumping dried liquid carbon dioxide into the vessel. The vessel was then stirred in a 35°C water bath for 3h. After 3h, the reaction vessel was cooled to room temperature and slowly de-gassed. The vessel contents and residue was washed three times with diethyl ether and filtered. Gas chromatography analysis revealed 84% conversion to phenylacetylcarbinol. Chiral gas chromatography (GC) showed a ratio of 87:13, 74% ee.

<sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.30 - 7.55, m, Ph; δ 5.15, s, CH; δ 2.10, s, CH<sub>3</sub>.

**Example 5:**      Reaction in liquid carbon dioxide

Benzaldehyde (0.137g, 1.3mmol), sodium pyruvate (2.1685g, 19.7mmol), pH 6 citrate buffer (5.4ml) and yeast (5.4g) were placed into a 250ml stainless steel pressure vessel. This vessel was pressurised to 1500 psi by pumping dried liquid carbon dioxide into the vessel. The vessel was then stirred at room temperature for 3h. After 3h, the reaction vessel was slowly de-gassed. The vessel contents and residue was washed three times with diethyl ether and filtered. Gas chromatography analysis revealed 84% conversion to phenylacetylcarbinol. Chiral GC showed a ratio of 95:5, 90% ee.

**Example 6:**      The yeast-mediated acyloin condensation of benzaldehyde using pyruvic acid

**(a)      Reaction in supercritical carbon dioxide**

Benzaldehyde (0.137g, 1.3mmol), yeast (5.4g) plus a mixture of pyruvic acid (0.216g, 2.45mmol) and water (5.4ml), buffered to pH = 5.45 using ammonium acetate, was placed into a 250ml stainless steel pressure vessel. This vessel was pressurised to 1500 psi by pumping dried liquid carbon dioxide into the vessel. The vessel was then stirred in a 35°C water bath for 3h. After 3h, the reaction vessel was cooled to room temperature and slowly de-gassed. The vessel contents and residue was washed three times with diethyl ether and filtered. Gas chromatography analysis revealed 44% conversion to phenylacetylcarbinol. Chiral GC showed a ratio of 96:4, 92% ee.

**(b)      Reaction in liquid carbon dioxide**

Benzaldehyde (0.137g, 1.3mmol), yeast (5.4g) plus a mixture of pyruvic acid (0.216g, 2.45mmol) and water (5.4ml), buffered to pH = 5.45 using ammonium acetate, were placed into a 250ml stainless steel pressure vessel. This vessel was pressurised to 1500 psi by pumping dried liquid

carbon dioxide into the vessel. The vessel was then stirred at room temperature for 3h. After 3h, the reaction vessel was slowly de-gassed. The vessel contents and residue was washed three times with diethyl ether and filtered. Gas chromatography analysis revealed 51% conversion to phenylacetylcarbinol. Chiral GC showed a ratio of 97:3, 94% ee.

**(c) Reaction in Liquefied Petroleum Gas**

Benzaldehyde (0.137g, 1.3mmol), yeast (5.4g) plus a mixture of pyruvic acid (0.216g, 2.45mmol) and water (5.4ml), buffered to pH = 5.45 using ammonium acetate, were placed into a 250ml stainless steel pressure vessel. This vessel was pressurised to 100 psi using liquefied petroleum gas (LPG). The vessel was then stirred at room temperature for 24h. After 24h, the reaction vessel was slowly de-gassed. The vessel contents and residue was washed three times with diethyl ether and filtered. Gas chromatography analysis revealed 12% conversion to phenylacetylcarbinol.

**(d) Reaction in Liquefied Petroleum Gas with the Addition of Ethanol**

Benzaldehyde (0.137g, 1.3mmol, yeast (5.4g), ethanol (0.54ml) plus a mixture of pyruvic acid (0.216g, 2.45mmol) and water (5.4ml), buffered to pH = 5.45 using ammonium acetate, were placed into a 250ml stainless steel pressure vessel. This vessel was pressurised to 100 psi using LPG. This vessel was then stirred at room temperature for 24h. After 24h, the reaction vessel was slowly de-gassed. The vessel contents and residue was washed three times with diethyl ether and filtered. Gas chromatography analysis revealed 14% conversion to phenylacetylcarbinol.

**(e) Phenylacetylcarbinol Extraction using Supercritical Carbon Dioxide**

A typical reaction mixture obtained from the reaction in carbon dioxide was extracted using carbon dioxide at a pressure of 2009 psi and a temperature of 40°C for a duration of 10 minutes. Subsequent gas chromatography analysis indicated that the phenylacetylcarbinol had been successfully isolated from the original reaction mixture.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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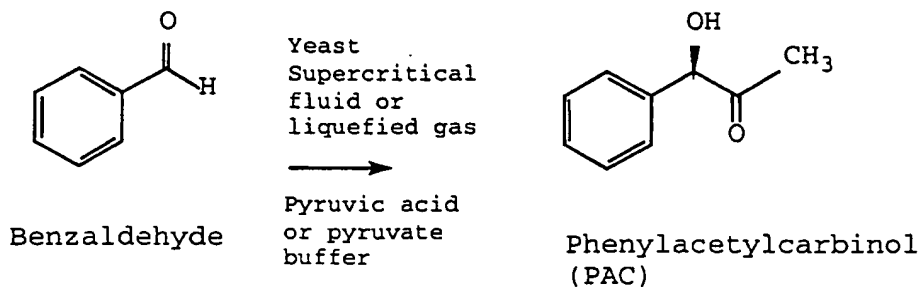


CLAIMS:

1. A method of synthesis of a substituted or unsubstituted carbinol compound, comprising the steps of  
5   subjecting the corresponding substituted or unsubstituted aromatic aldehyde to acyloin condensation mediated by yeast in the presence of either
  - (a) a supercritical fluid or
  - (b) a liquefied gas,  
10   and recovering the carbinol compound.
2. A method according to claim 1, in which the yeast is *Saccharomyces cerevisiae*.
- 15   3. A method according to claim 1 or claim 2, in which the yeast is specifically adapted to a supercritical fluid environment or for enhanced acyloin condensation efficiency.
- 20   4. A method according to any one of claims 1 to 3, in which the maximum surface area of yeast is presented for contact with the reactants.
- 25   5. A method according to any one of claims 1 to 4, in which the condensation is performed in the presence of one or more liquefied gases selected from the group consisting of carbon dioxide, methane, ethane, ethylene, propane, butane and liquefied petroleum gas.
- 30   6. A method according to any one of claims 1 to 4, in which the condensation is performed in the presence of a supercritical fluid, and the carbinol compound is recovered by subjecting the reaction mixture to extraction with a supercritical fluid or an organic solvent.
- 35   7. A method according to claim 5, in which the supercritical fluid is selected from the group consisting

of carbon dioxide, ethane, nitrous oxide, xenon, fluoroform, monofluoromethane, sulphur hexafluoride and chlorotrifluoromethane.

- 5    8.            A method according to claim 7, in which the supercritical fluid is carbon dioxide.
9.            A method according to claim 8, in which the condensation is performed at a temperature of 33 to 42°C.
- 10    10.           A method according to claim 9, in which the condensation is performed at a temperature of 35°C.
11.           A method according to any one of claims 8 to 10,
- 15    in which the condensation is performed at a pressure of 1500 to 2500 psi.
12.           A method according to claim 9, in which the condensation is performed at a pressure of 2500 psi.
- 20    13.           A method according to any one of claims 1 to 12, in which the condensation is performed in the presence of an inhibitor of side-product formation.
- 25    14.           A method according to claim 13, in which the inhibitor is ethanol.
15.           A method according to any one of claims 1 to 14, in which the aromatic aldehyde is benzaldehyde and the
- 30    carbinol is phenylacetylcarbinol, according to the following reaction:



in which the benzaldehyde, the pyruvic acid, or both may  
5 optionally be substituted.

16. A method according to claim 15, in which the  
benzaldehyde is substituted with one or more alkyl, aryl,  
halo, nitro, hydroxy, alkoxy, amino, carbonyl, thioxy or  
10 thioalkoxy groups, or composites of these groups.

17. A method according to claim 15 or claim 16, in  
which the pH of the pyruvate/citrate buffer solution is  
between 5 and 6.

15

18. A method according to any one of claims 1 to 17,  
in which benzaldehyde is converted to phenylacetylcarbinol,  
0.6 to 1.2 ml buffer/g is used, and the ratio of yeast to  
substrate is 4.2 g yeast/mmol benzaldehyde.

20

19. A method according to claim 18, in which the  
reaction time is 3 to 24 hours.

20. A method according to any one of claims 15 to 19,  
25 in which the supercritical fluid is not carbon dioxide, and  
a precursor of pyruvic acid which is converted *in situ* to  
pyruvic acid is used.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU00/01543

**A. CLASSIFICATION OF SUBJECT MATTER**Int. Cl. <sup>7</sup>: C12P 7/26, 7/28, C07C 45/45, 49/245

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: (CAOLD ; CA; WPIDS) Keywords: acyloin; condensation;  
Yeast ; Saccharomyces ; supercritical ; liquified.**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Prog. Biotechnol. (1992), 8 (Biocatalysis in Non-Conventional Media); Isenschmid, A et al; Effect of a near-critical and supercritical fluid on .....cells; pages 407-16 See page 407, abstract.	1-20
Y	J. Chem. Soc., Perkin Trans. 1 (1996, (5); Bornemann, Stephen et al; Stereospecific formation.....decarboxylase; pages 425-30 See whole document .	1-20
Y	Appl. Microbiol. Biotechnol (1991), 36(3); Cardillo, Rosanna et al.; Biotransformation of unsaturated.....activity; pages 300-3. See whole document .	1-20

☒ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

31 January 2001

Date of mailing of the international search report

8 February 2001

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU00/01543

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J. Chem. Soc., Chem. Commun. (1993), (4); Kren, Vladimir et al.; Pyruvate decarboxylase: a new.....biotransformation; pages 341-3. See whole document.	1-20
Y	US 5173413 A (Robert W. Coughlin et al.) 22 December 1992. See whole document.	1-20
Y	WO 90/04639 A (Synergen, Inc.) 3 May 1990. See whole document	1-20
Y	WO 99/63103 A (Victoria University of Technology). 9 December 1999. See whole document.	1-20

INTERNATIONAL SEARCH REPORT  
Information on patent family members

International application No.  
PCT/AU00/01543

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report			Patent Family Member				
US	5173413	NONE					
WO	9004639	AU	45019/89	CA	2000721	EP	439527
WO	9963103	AU	42498/99				
							END OF ANNEX

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